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(54) Ascorbic acid 2-phosphate zinc salt and process for manufacturing the same

(57) L-Ascorbic acid 2-phosphate zinc salt and a salt hydrate thereof having excellent solubility and exhibiting good stability even under weakly acidic conditions. Also disclosed is a process of manufacturing L-ascorbic acid 2-phosphate zinc salt by displacing a cation of a salt of an L-ascorbic acid 2-phosphate other than a zinc salt with a zinc cation. Further disclosed is a composition containing L-ascorbic acid 2-phosphate zinc salt or a salt hydrate thereof as an active ingredient.

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Description**FIELD OF THE INVENTION**

5 The present invention relates to a novel L-ascorbic acid 2-phosphate salt, a process for manufacturing the salt and a method of use thereof. More specifically, the present invention relates to L-ascorbic acid 2-phosphate zinc salt or a salt hydrate of zinc L-ascorbic acid 2-phosphate (hereinafter, unless otherwise indicated, the salt including the salt hydrate thereof is referred to as an "L-ascorbic acid 2-phosphate zinc salt") which is a soluble L-ascorbic acid 2-phosphate salt having improved stability. L-ascorbic acid 2-phosphate zinc salt does not cause an extreme alkaline condition
10 on dissolving in water and also exhibits antimicrobial activity. The present invention also relates to a method for manufacturing the salt and a method of use thereof. By using this salt, an L-ascorbic acid 2-phosphate-containing preparation (e.g., a cosmetic preparation, medical preparation, agricultural chemical preparation, animal drug preparation, foodstuff preparation and feedstuff preparation) having improved solubility, stability and antimicrobial properties (including anti-septic, deodorizing and antidandruff effects) and which is stable under weakly acidic conditions, can be obtained.

BACKGROUND OF THE INVENTION

20 L-Ascorbic acid (vitamin C) imparts many physiological effects. For example, vitamin C restrains lipid peroxide, accelerates the formation of collagen, retards the formation of melanin and enhances immune functions, and has hitherto been used to achieve these effects in the fields of medicine, agricultural chemicals, animal drugs, foodstuffs, feedstuffs and cosmetic preparations. Many preparations containing L-ascorbic acid are commercially available. However, L-ascorbic acid has poor aging stability, and the effect of vitamin C cannot be always obtained even if such a preparation is used.

25 In order to improve stability, derivatives obtained by converting the hydroxyl groups in the enediol moiety at the 2- or 3-position each into a glycosyl group (for example, JP-A-5-117290 (the term "JP-A" as used herein means an "unexamined published Japanese patent application")) or into a phosphoric acid ester (for example, JP-B-52-18191 (the term "JP-B" as used herein means an "examined Japanese patent publication") and JP-A-2-279690) have been proposed.

30 However, L-ascorbic acid 2-glycoside salts are converted into L-ascorbic acid in vivo (particularly in humans) at a low rate as compared with L-ascorbic acid 2-phosphates. Therefore, a prompt effect cannot be achieved and an adequately high vitamin C effect can hardly be obtained.

35 L-Ascorbic acid 2-phosphates provide a prompt effect, and an adequately high vitamin C effect can be easily obtained. However, conventionally known L-ascorbic acid 2-phosphates are a magnesium salt, a calcium salt and a sodium salt, and among these salts, L-ascorbic acid 2-phosphate magnesium salt having excellent stability does not have sufficiently high solubility in water and is difficult to form into an aqueous solution having a concentration of 30% or more. The calcium salt scarcely dissolves in water.

40 Sodium L-ascorbic acid 2-phosphate having satisfactorily improved solubility can be easily formed into an aqueous solution having a concentration of about 30%, but does not have a stability that is comparable with that of the magnesium salt. Furthermore, when dissolving any of these salts, the pH of the resulting solution is in an alkaline region of approximately from 8 to 10. When the pH is adjusted to a weakly acidic condition, the stability is disadvantageously lowered.

45 Although the above-described L-ascorbic acid derivatives have been proposed, an L-ascorbic acid 2-phosphate having excellent solubility and which is sufficiently stable even under weakly acidic conditions has not yet been obtained.

50 In obtaining a salt in general, neutralization of an acid or base is used in many cases. However, this method is not appropriate for manufacturing an L-ascorbic acid 2-phosphate salt on an industrial scale. This is because an L-ascorbic acid 2-phosphate solution is strongly acidic and unstable.

SUMMARY OF THE INVENTION

55 It is therefore an object of the present invention to provide an L-ascorbic acid 2-phosphate having excellent solubility and which exhibits sufficiently improved stability even under weakly acidic conditions.

Under these circumstances, the present inventors have conducted extensive investigations and as a result, have found that hitherto unknown L-ascorbic acid 2-phosphate zinc salt is a compound having highly improved solubility and stability even under weakly acidic conditions, and also provides an antimicrobial (antiseptic) effect.

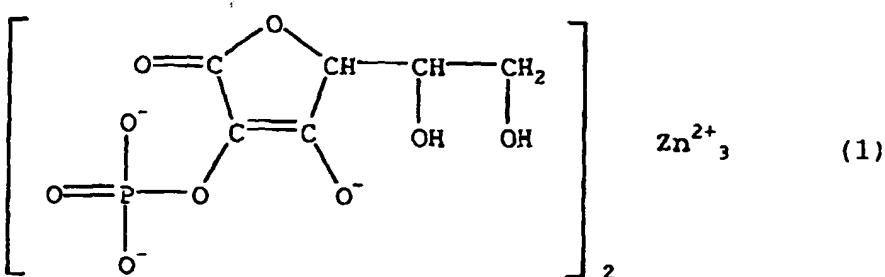
55 The above object of the present invention has been achieved by providing L-ascorbic acid 2-phosphate zinc salt and a salt hydrate of zinc L-ascorbic acid 2-phosphate which, in addition to the above properties, has also been found to have an antimicrobial effect. The present invention further provides a process for manufacturing the L-ascorbic acid 2-phosphate zinc salt and salt hydrate thereof and a method of using the salt and salt hydrate thereof in cosmetic and

medical preparations, agricultural chemical preparations, animal drug preparations, foodstuff preparations and feedstuff preparations so as to increase the action of vitamin C or to provide an antimicrobial, antiseptic, deodorizing or antian-
druff effect.

5 **DESCRIPTION OF THE PREFERRED EMBODIMENTS**

Examples of the process for manufacturing the compound of the present invention include a manufacturing process starting from a soluble L-ascorbic acid 2-phosphate salt and using an ion exchange technique or the like, and a combination of this manufacturing process with other purification methods, as needed. However, the present invention is by
10 no means limited thereto.

If L-ascorbic acid 2-phosphoric acid is represented by ApH_3 , the zinc salt of the present invention is represented by Ap_2Zn_3 and has a structure as shown in formula (1) below:



Ap_2Zn_3 is white powder and can be obtained as an anhydride or salt hydrate. The salt hydrate is considered to be a hydrate $Ap_2Zn_3 \cdot nH_2O$ of Ap_2Zn_3 .

In a preferred embodiment of the L-ascorbic acid 2-phosphate zinc salt or salt hydrate thereof of the present invention, 95 mol% or more of the entire cationic charge constitutes zinc ion and 95 mol% or more of the entire anionic charge constitutes L-ascorbic acid 2-phosphate ion. The Zn cation and Ap anion are present in sufficient amounts if each is contained in total in the above-described amounts. For example, when Ap_2Zn_3 is present in an amount of 95 mol% or more of the total L-ascorbic acid 2-phosphate salt content, Ap_2Zn_2Mg or the like may be present in part. However, this is acceptable if the zinc ion content is 95 mol% or more of the total cation content.

35 The L-ascorbic acid 2-phosphate zinc salt of the present invention may be manufactured by a process using, for example, a soluble L-ascorbic acid 2-phosphate salt other than the zinc salt, such as Ap_2Mg_3 , as a starting material and replacing the magnesium with zinc by chemical reaction or using an ion exchange technique (e.g., treating with an ion exchange resin). This process may also be combined with a purification method. Examples of the chemical reaction include:



In a manufacturing process using an ion exchange technique, the soluble L-ascorbic acid 2-phosphate salt used as a starting material is not necessarily purified, but may be, for example, an L-ascorbic acid 2-phosphate-containing solution obtained by reacting L-ascorbic acid with phosphorus oxychloride. The ion exchange step may employ, for example, cation exchange column chromatography or anion exchange chromatography.

When using cation exchange column chromatography, an aqueous L-ascorbic acid 2-phosphate zinc salt solution may be eluted by passing an L-ascorbic acid 2-phosphate solution through a column containing a zinc type cation exchange resin (preferably a strong cationic exchange resin).

50 In the case of using anion exchange chromatography, a solution containing L-ascorbic acid 2-phosphate is passed through the anion exchange resin column to adsorb L-ascorbic acid 2-phosphate ion onto the resin, a zinc salt solution is passed therethrough, and the L-ascorbic acid 2-phosphate zinc salt fraction is collected.

In the case where the L-ascorbic acid 2-phosphate solution that is added to the column contains a substance which forms a zinc salt having low solubility, the solution is previously processed to remove such substances (for example, in 55 the case of phosphorus ion, a zinc salt or magnesium salt is added to precipitate and remove the substance). In this manner, the column chromatography can be performed more efficiently.

The chromatography conditions (temperature, flow rate, composition of added solution, concentration, amount of solution, etc.) may be established by taking into account general column chromatography parameters (e.g., column

size, physical properties of the resin, exchange capacity, separation capability, viscosity of the added solution, solubility, etc.) well known to those of ordinary skill in the art.

L-ascorbic acid 2-phosphate zinc salt powder can be obtained directly from the above solution containing L-ascorbic acid 2-phosphate zinc salt, or by precipitating or the like in an organic solvent and removing the solvent by spray drying, vacuum drying or freeze drying. When the L-ascorbic acid 2-phosphate zinc salt powder is obtained by precipitating in an organic solvent, the zinc salt added to the column in the anion exchange method preferably, in view of purity, is a salt which is highly soluble in the organic solvent (for example, in the case of using acetone as the organic solvent, zinc chloride or zinc propionate may be used).

The thus-obtained L-ascorbic acid 2-phosphate zinc salt has excellent solubility and is easily formed into an aqueous solution having a concentration of 50 wt% or more. Furthermore, the L-ascorbic acid 2-phosphate zinc salt of the present invention is remarkably stable even under weakly acidic conditions, and a composition containing an L-ascorbic acid 2-phosphate having sufficiently improved stability even in such a pH region can be obtained. Furthermore, a composition also having an antimicrobial effect (including antiseptic, deodorizing and antidandruff effects) can be obtained. Furthermore, vitamin C can be effectively supplied by administering the composition to humans or animals. Examples of the composition include cosmetic preparations, medical preparations, agricultural chemical preparations, animal drug preparations, foodstuff preparations and feedstuff preparations. The ingredients of the composition and the amount of the L-ascorbic acid 2-phosphate zinc salt added thereto are the same as in the case of using a conventional L-ascorbic acid 2-phosphate salt.

For example, a general and preferred range for the content (wt%) of the L-ascorbic acid 2-phosphate zinc salt when added to a cosmetic preparation, a medical preparation, an agricultural chemical preparation, an animal drug preparation, a foodstuff preparation and a feedstuff preparation may be as follows:

Preparation Type	General	Preferred
Cosmetic preparation	0.01 - 50%	0.1 - 5%
Medical preparation	0.01 - 50%	0.1 - 20%
Agricultural chemical preparation	0.01 - 50%	0.1 - 10%
Animal drug preparation	0.01 - 50%	0.1 - 20%
Foodstuff preparation	0.001 - 50%	0.002 - 5%
Feedstuff preparation	0.001 - 50%	0.002 - 5%

In the case of a salt hydrate, the above amounts are increased to take into account the water of hydration.

EXAMPLES

The present invention is described in greater detail below by reference to the following Examples, however, the present invention should not be construed as being limited thereto.

In the Examples and Test Examples, the analysis of an L-ascorbic acid 2-phosphate salt or an ion thereof was performed using an Ap-containing solution having a known concentration (prepared from L-ascorbic acid 2-phosphate magnesium salt) as a standard by an HPLC method using a Shodex IEC DEAE-825 resin (ammonium acetate 10 mM → 1M gradient, detected spectrophotometrically at 265 nm). The elemental analysis was performed by an ICP emission method, flame photometry and an ion chromatography method. Unless otherwise indicated, the reagents used herein were commercially available reagents.

Example 1

Under a nitrogen atmosphere, 10 g of L-ascorbic acid was dissolved in a mixed solvent of 135 ml of water and 15 g of pyridine, and the resulting mixed solution was cooled to 0°C and adjusted to a pH of 11.5 with a 12% (by weight, hereinafter the same) potassium hydroxide solution. While keeping the solution at a temperature of from 0 to 5°C, 15 g of phosphorus oxychloride was added by means of a quantitative pump. During the addition, the pH was kept within a range of from 11.45 to 11.55 with a 12% potassium hydroxide solution. After completing the addition, the mixture was stirred at the same temperature for 10 minutes, and 70 ml of a 1.5M magnesium chloride solution was gradually added thereto. The resulting solution was further stirred for 30 minutes, and the precipitate was then removed by suction filtra-

tion.

The solution thus obtained was added to a column containing an OH-type anion exchange resin Diaion SA 10A (trade name, manufactured by Mitsubishi Kasei KK) having a column size of $2.5\text{ cm}\varnothing \times 50\text{ cm}$, and washed with 1 L of water. The running direction of the solution was reversed, 250 ml of a 3M zinc chloride solution was added at a rate of 5 10 ml/min, and 250 ml of an eluent was obtained. To the eluent, 1 L of acetone was gradually added, and the mixture was stirred for 30 minutes. The precipitate was collected by suction filtration, washed three times with 200 ml of acetone, and vacuum dried to obtain 4.3 g of powder. A small portion of the powder was dissolved in purified water in an amount of 100 ppm and analyzed by the HPLC method described above. As a result, the solution was found to contain Ap in a concentration of 56.7 ppm. Namely, the powder contained Ap in an amount of 56.7%. The elemental analysis 10 for the powder was as follows. C: 16.2%, H: 3.6%, O: 50.2%, P: 7.0%, Zn: 22.3%, Mg: 0.1%, Cl: 0.4%, and K: <0.1%. Thus, the powder was verified to have a purity of 95% or more as $\text{Ap}_2\text{Zn}_3 \cdot 10\text{H}_2\text{O}$.

Example 2

15 To a cation exchange column containing the resin Diaion SK 1B (trade name, manufactured by Mitsubishi Kagaku KK) having a column size of $5\text{ cm}\varnothing \times 25\text{ cm}$, 1.5 L of 1M sulfuric acid, 0.5 L of water, 1L of 1M zinc sulfate and 2 L of water were added in sequence at a rate of 20 ml/min to form a zinc type resin.

To the column, 0.5 L of a 10% aqueous solution of L-ascorbic acid PM (L-ascorbic acid 2-phosphate magnesium salt, trade name, produced by Showa Denko KK) and 0.5 L of water were added in sequence at a rate of 10 ml/min, and 20 1 L of eluent was collected. The eluent thus collected was freeze dried to obtain 52 g of powder. A small portion of this powder was dissolved in purified water in an amount of 100 ppm, and the resulting solution was analyzed by the HPLC method described above. As a result, the solution was found to contain Ap in a concentration of 58.6 ppm. Namely, the powder obtained contained Ap in an amount of 58.6%. The elemental analysis for the powder was as follows. C: 16.7%, H: 3.5%, O: 50.1%, P: 7.2%, Zn: 22.5%, and Mg: <0.1%. Thus, the powder was verified to have a purity of 99% or more 25 as $\text{Ap}_2\text{Zn}_3 \cdot 9\text{H}_2\text{O}$.

Unless otherwise indicated, in Test Examples 1 and 2 and in Example 3 below, the L-ascorbic acid 2-phosphate zinc salt obtained in Examples 1 and 2 was used in the following evaluations.

Test Example 1 - Stability

30 The stability of L-ascorbic acid 2-phosphate zinc salt was compared to that of L-ascorbic acid 2-phosphate magnesium salt. Each salt was dissolved in a buffer solution (50 mM acetic acid-NaOH, pH: 5, or 50 mM Tris-HCl, pH: 8) to a concentration of about 0.1% and stored at 50°C for 476 hours. The concentrations of the L-ascorbic acid 2-phosphate before and after storage were analyzed by the HPLC method.

35

Table 1

L-ascorbic acid 2-phosphate	Stability (100% = concentration before storage)	
	pH: 5	pH: 8
L-ascorbic acid 2-phosphate zinc salt (Example 1)	91	93
L-ascorbic acid 2-phosphate zinc salt (Example 2)	92	95
L-ascorbic acid 2-phosphate magnesium salt	82	95

40 The above results show that the stability of the L-ascorbic acid 2-phosphate zinc salt of the present invention is hardly reduced even when stored under weakly acidic conditions.

50

Test Example 2 - Antimicrobial effect

To NUTRIENT BROTH (produced by DIFCO), 0.1% of an L-ascorbic acid 2-phosphate salt was added thereto, and the pH was adjusted to 6.8 using hydrochloric acid or sodium hydroxide. Using 5 ml of the medium thus prepared, test 55 bacterium were inoculated in a concentration of about 10^7 cells/ml and incubated in a test tube at 37°C for 24 hours. The results are shown in Table 2 below. In Table 2, "+" indicates that turbidity increased after incubation, and "-" indicates no increase in turbidity. As a control, the same test was performed using a medium not containing an L-ascorbic acid 2-phosphate salt.

Table 2

L-Ascorbic acid 2-phosphate	Test bacterium	
	<i>E. coli</i> (K-12 strain)	<i>B. subtilis</i> (168 strain)
Zinc salt (Example 1)	-	-
Zinc salt (Example 2)	-	-
Magnesium salt	+	+
Sodium salt	+	+
Not added	+	+

The above results show that the L-ascorbic acid 2-phosphate zinc salt of the present invention has antimicrobial properties.

20 Example 3 - Cosmetic Preparation

One example of a cosmetic preparation in accordance with the present invention is shown below.

Table 3

	Ingredient	% by weight
(A)	Liquid paraffin	5.0
	Hexalan (produced by Kyoei Kagaku Kogyo KK)	3.0
	Paraffin	4.0
	Beeswax HP (produced by Kyoei Kagaku Kogyo KK)	4.0
	Sorbitan monostearate	1.0
	Polyoxyethylene (5) sorbitan monostearate	3.0
	Glycerin monostearate	1.0
(B)	Glycerin	4.0
	L-ascorbic acid 2-phosphate zinc salt (Example 1)	3.0
	β -Cyclodextrin	1.0
	Water	70.8
(C)	Perfume	0.2

45 Ingredient (A) and Ingredient (B) each was heated at 80°C, then mixed and stirred. After cooling to 50°C, Ingredient (C) was added, and the ingredients were mixed by further stirring to prepare a homogeneous cream.

50 Example 4 - Medical Preparations

(1) Preparation for an injectable drug (e.g., a drug for supplementing vitamin C)

L-ascorbic acid 2-phosphate zinc salt (Example 1) glucose	1.0 wt% 5 wt%
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(continued)

distilled water	94 wt%
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5 The above ingredients were dissolved in distilled water and sterilized by filtering through a membrane filter with a pore size of 0.2 µm.

(2) Preparation for an oral capsule drug (e.g., an antiulcer drug)

10

L-ascorbic acid 2-phosphate zinc salt (Example 1)	10 wt%
corn starch	85 wt%
magnesium stearate	5 wt%

15

The above ingredients were thoroughly mixed using a mill, and 100 mg of the mixture was inserted into a gelatin capsule.

20

(3) Preparation for an oral powder drug (e.g., an antiulcer drug)

25

L-ascorbic acid 2-phosphate zinc salt (Example 1)	10 wt%
crystalline lactose	90 wt%

30

The above ingredients were thoroughly mixed using a mill.

35

(4) Preparation for an ointment drug (e.g., a drug for acne treatment)

40

L-ascorbic acid 2-phosphate zinc salt (Example 1)	5 wt%
squalene	10 wt%
stearic acid	10 wt%
propyleneglycol monostearate	3 wt%
polyoxyethylene cetyl ether	1 wt%
propyleneglycol	15 wt%
methyl p-hydroxybenzoate	0.2 wt%
distilled water	55.8 wt%

45

The above ingredients were mixed and stirred at 80°C to obtain a homogeneous ointment.

50 Example 5 - Feedstuff Preparations

(1) Preparation for a feed additive for cattle, chicken, dogs and cats (e.g., a vitamin C supplement)

55

L-ascorbic acid 2-phosphate zinc salt (Example 1)	0.06 wt%
wheat bran	30 wt%

(continued)

barley meal	20 wt%
rice bran	42 wt%
soybean meal	5 wt%
sodium chloride	0.5 wt%
vitamin mixture (not containing vitamin C)	2.44 wt%

5

10 The above ingredients were mixed and pelleted using a pelletter at 80°C and dried at 100°C.

(2) Preparation for a feed additive for fish (e.g., a vitamin C supplement)

15

L-ascorbic acid 2-phosphate zinc salt (Example 1)	0.01 wt%
fish meal	56 wt%
salmon milt	6 wt%
squid liver oil	5 wt%
soybean lecithin	4 wt%
valine	0.3 wt%
isoleucine	0.2 wt%
corn starch	5 wt%
mineral mixture	5 wt%
gluten	8 wt%
vitamin mixture (not containing vitamin C)	2.59 wt%
ω3-HUFA	1 wt%
soybean meal	6.9 wt%

20

25

30

35

The above ingredients were mixed and pelleted using a pelletter at 80°C and dried at 100°C.

Example 6 - Foodstuff Preparation

40

(1) Preparation for a food additive (e.g., a vitamin C supplement)

45

L-ascorbic acid 2-phosphate zinc salt (Example 1)	10 wt%
citric acid disodium salt	0.5 wt%
oligosugar	89.5 wt%

50

The above ingredients were thoroughly mixed using a mill.

The L-ascorbic acid 2-phosphate zinc salt of the present invention is highly soluble and stable under weakly acidic conditions, and also has antimicrobial properties. By adding the salt to cosmetic preparations, medical preparations, agricultural chemical preparations, animal drug preparations, foodstuff preparations and feedstuff preparations, a vitamin C-containing preparation having improved solubility and stability and having an antimicrobial effect can be obtained.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from

the spirit and scope thereof.

Claims

- 5 1. L-Ascorbic acid 2-phosphate zinc salt.
2. A salt hydrate of zinc L-ascorbic acid 2-phosphate.
- 10 3. The L-ascorbic acid 2-phosphate zinc salt as claimed in claim 1 or 2, wherein 95 mol% or more of the entire cationic charge of said salt constitutes zinc ion and 95 mol% or more of the entire anionic charge of said salt constitutes L-ascorbic acid 2-phosphate ion.
- 15 4. A process for manufacturing L-ascorbic acid 2-phosphate zinc salt, which comprises displacing a cation of a salt of an L-ascorbic acid 2-phosphate other than a zinc salt with a zinc cation.
- 20 5. The process for manufacturing L-ascorbic acid 2-phosphate zinc salt as claimed in claim 4, wherein 95 mol% or more of the entire cationic charge of said salt constitutes zinc ion and 95 mol% or more of the entire anionic charge of said salt constitutes L-ascorbic acid 2-phosphate ion.
- 25 6. A composition comprising L-ascorbic acid 2-phosphate zinc salt or a salt hydrate of zinc L-ascorbic acid 2-phosphate as an active ingredient.
7. The composition as claimed in claim 6, wherein 95 mol% or more of the entire cationic charge of said salt or salt hydrate constitutes zinc ion and 95 mol% or more of the entire anionic charge of said salt or salt hydrate constitutes L-ascorbic acid 2-phosphate ion.
- 30 8. The composition as claimed in claim 6, wherein said composition is selected from the group consisting of a cosmetic preparation, a medical preparation, an agricultural chemical preparation, an animal drug preparation, a foodstuff preparation and a feedstuff preparation.
9. A vitamin C agent comprising L-ascorbic acid 2-phosphate zinc salt or a salt hydrate of zinc L-ascorbic acid 2-phosphate as an active ingredient.
- 35 10. The composition as claimed in claim 6, containing L-ascorbic acid 2-phosphate zinc salt in an amount of from 0.01-50 wt% when the composition is a cosmetic preparation, a medical preparation, an agricultural chemical preparation or an animal drug preparation, or in an amount of from 0.001-50 wt% when the composition is a foodstuff preparation or a feedstuff preparation.

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EUROPEAN SEARCH REPORT

Application Number

EP 98 11 0676

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	FR 1 489 249 A (TAKEDA CHEMICAL IND.) 27 October 1967 * the whole document *	1-10	C07F9/655 A23L3/3544 A23L3/3553 A61K31/665 A61K7/00 A23K3/00
A	US 3 671 549 A (DAVID F. HINKLEY) 20 June 1972 * the whole document *	1-10	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C07F A23L A61K A23K
Place of search			Date of completion of the search
THE HAGUE		11 September 1998	Examiner
Beslier, L			
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